FILE 'REGISTRY' ENTERED AT 10:47:45 ON 28 JUL 2003

=> S 2,5-DIKETO-D-GLUCONIC ACID PERMEASE/CN
L1 0 2,5-DIKETO-D-GLUCONIC ACID PERMEASE/CN

=> S 2,5-DIKETO-D-GLUCONIC ACID PERMEASE
576644 2,5
428 DIKETO
3894427 D
2079 GLUCONIC
5999361 ACID
8307 ACIDS
6005516 ACID
(ACID OR ACIDS)
5726 PERMEASE
41 PERMEASES
5726 PERMEASE
(PERMEASE OR PERMEASES)
L2 0 2,5-DIKETO-D-GLUCONIC ACID PERMEASE
(2,5(W)DIKETO(W)D(W)GLUCONIC(W)ACID(W)PERMEASE)

=> S DIKETO GLUCONIC ACID PERMEASE/CN
L3 0 DIKETO GLUCONIC ACID PERMEASE/CN

=> S DIKETO GLUCONIC ACID PERMEASE
428 DIKETO
2079 GLUCONIC
5999361 ACID
8307 ACIDS
6005516 ACID
(ACID OR ACIDS)
5726 PERMEASE
41 PERMEASES
5726 PERMEASE
(PERMEASE OR PERMEASES)
L4 0 DIKETO GLUCONIC ACID PERMEASE
(DIKETO(W)GLUCONIC(W)ACID(W)PERMEASE)

=> S 2,5-DI-KETO-D-GLUCONIC ACID PERMEASE/CN
L5 0 2,5-DI-KETO-D-GLUCONIC ACID PERMEASE/CN

=> S 2,5-DI-KETO-D-GLUCONIC ACID PERMEASE
576644 2,5
11541340 DI
40384 DIS
11541340 DI
(DI OR DIS)
4987 KETO
31 KETOS
4987 KETO
(KETO OR KETOS)
3894427 D
2079 GLUCONIC
5999361 ACID
8307 ACIDS
6005516 ACID
(ACID OR ACIDS)
5726 PERMEASE
41 PERMEASES
5726 PERMEASE
(PERMEASE OR PERMEASES)
L6 0 2,5-DI-KETO-D-GLUCONIC ACID PERMEASE
(2,5(W)DI(W)KETO(W)D(W)GLUCONIC(W)ACID(W)PERMEASE)

=> S DI-KETO GLUCONIC ACID PERMEASE
11541340 DI
40384 DIS
11541340 DI
(DI OR DIS)
4987 KETO
31 KETOS

```
          4987 KETO
                 (KETO OR KETOS)
          2079 GLUCONIC
       5999361 ACID
          8307 ACIDS
       6005516 ACID
                 (ACID OR ACIDS)
          5726 PERMEASE
            41 PERMEASES
          5726 PERMEASE
                 (PERMEASE OR PERMEASES)
L7           0 DI-KETO GLUCONIC ACID PERMEASE
                 (DI(W)KETO(W)GLUCONIC(W)ACID(W)PERMEASE)

FILE 'CAPLUS' ENTERED AT 10:49:50 ON 28 JUL 2003

=> S 2,5-DIKETO-D-GLUCONIC ACID PERMEASE
       7782983 2
       5454920 5
          2566 DIKETO
             2 DIKETOS
          2568 DIKETO
                 (DIKETO OR DIKETOS)
       2004706 D
          8118 GLUCONIC
       3671955 ACID
       1390456 ACIDS
       4134012 ACID
                 (ACID OR ACIDS)
          2914 PERMEASE
           676 PERMEASES
          3148 PERMEASE
                 (PERMEASE OR PERMEASES)
L8           0 2,5-DIKETO-D-GLUCONIC ACID PERMEASE
                 (2(W)5(W)DIKETO(W)D(W)GLUCONIC(W)ACID(W)PERMEASE)

=> S (DIKETO OR DI-KETO)(W)(GLUCONIC ACID PERMEASE)
          2566 DIKETO
             2 DIKETOS
          2568 DIKETO
                 (DIKETO OR DIKETOS)
        427624 DI
          3962 DIS
        431431 DI
                 (DI OR DIS)
         51939 KETO
             6 KETOS
         51945 KETO
                 (KETO OR KETOS)
            41 DI-KETO
                 (DI(W)KETO)
          8118 GLUCONIC
       3671955 ACID
       1390456 ACIDS
       4134012 ACID
                 (ACID OR ACIDS)
          2914 PERMEASE
           676 PERMEASES
          3148 PERMEASE
                 (PERMEASE OR PERMEASES)
             0 GLUCONIC ACID PERMEASE
                 (GLUCONIC(W)ACID(W)PERMEASE)
L9           0 (DIKETO OR DI-KETO)(W)(GLUCONIC ACID PERMEASE)

=> S GLUCONIC ACID PERMEASE
          8118 GLUCONIC
       3671955 ACID
       1390456 ACIDS
       4134012 ACID
                 (ACID OR ACIDS)
          2914 PERMEASE
```

```
          676 PERMEASES
         3148 PERMEASE
                (PERMEASE OR PERMEASES)
L10         0 GLUCONIC ACID PERMEASE
                (GLUCONIC(W)ACID(W)PERMEASE)

=> S "2,5"(W)DKG
      7782983 "2"
      5454920 "5"
       599910 "2,5"
                ("2"(W)"5")
          103 DKG
L11        29 "2,5"(W)DKG

=> S 2,5(W)DKG
      7782983 2
      5454920 5
       599910 2,5
                (2(W)5)
          103 DKG
L12        29 2,5(W)DKG

=> D 1-29 CBIB ABS

L12  ANSWER 1 OF 29  CAPLUS  COPYRIGHT 2003 ACS on STN
2003:99002   Document No. 138:237006  Mathematical modeling of in vitro
     enzymatic production of 2-keto-L-gulonic acid using NAD(H) or NADP(H) as
     cofactors.  Banta, Scott; Boston, Matt; Jarnagin, Alisha; Anderson,
     Stephen (Department of Chemical and Biochemical Engineering, Rutgers, The
     State University of New Jersey, Piscataway, NJ, 08854, USA).  Metabolic
     Engineering, 4(4), 273-284 (English) 2002.  CODEN: MEENFM.  ISSN:
     1096-7176.  Publisher: Elsevier Science.
AB   A 2-Keto-L-gulonic acid (2-KLG) prodn. process using stationary Pantoea
     citrea cells and a Corynebacterium 2,5-diketo-D-gluconic acid (  ***2***
     ,  ***5***  -  ***DKG***  ) reductase enzyme has been developed which may
     represent an improved method of vitamin C biosynthesis.  Exptl. data was
     collected using the F22Y/A272G   ***2***  ,  ***5***  -  ***DKG***
     reductase mutant and NADP(H) as a cofactor.  An extensive kinetic anal.
     was performed and a kinetic rate equation model for this process was
     developed.  A recent protein engineering effort has resulted in several
       ***2***  ,  ***5***  -  ***DKG***  reductase mutants exhibiting improved
     activity with NADH as a cofactor.  The use of NAD(H) in the bioreactor may
     be preferable due to its increased stability and lower cost.  The kinetic
     parameters in the rate equation model have been replaced in order to
     predict 2-KLG prodn. with NAD(H) as a cofactor.  The model was also
     extended to predict 2-KLG prodn. in the presence of a range of combined
     cofactor concns.  This anal. suggests that the use of the
     F22Y/K232G/R238H/A272G   ***2***  ,  ***5***  -  ***DKG***  reductase
     mutant with NAD(H) combined with a small amt. of NADP(H) could provide a
     significant cost benefit for in vitro enzymic 2-KLG prodn.

L12  ANSWER 2 OF 29  CAPLUS  COPYRIGHT 2003 ACS on STN
2002:963274   Document No. 138:149558  Verification of a novel NADH-binding
     motif: combinatorial mutagenesis of three amino acids in the
     cofactor-binding pocket of Corynebacterium 2,5-diketo-D-gluconic acid
     reductase.  Banta, Scott; Anderson, Stephen (Department of Chemical and
     Biochemical Engineering, Center for Advanced Biotechnology and Medicine,
     Rutgers, The State University of New Jersey, Piscataway, NJ, 08854, USA).
     Journal of Molecular Evolution, 55(6), 623-631 (English) 2002.  CODEN:
     JMEVAU.  ISSN: 0022-2844.  Publisher: Springer-Verlag New York Inc..
AB   A screening method has been developed to support randomized mutagenesis of
     amino acids in the cofactor-binding pocket of the NADPH-dependent
     2,5-diketo-D-gluconic acid (  ***2***  ,  ***5***  -  ***DKG***  )
     reductase.  Such an approach could enable the isolation of an enzyme that
     can better catalyze the redn. of   ***2***  ,  ***5***  -  ***DKG***   to
     2-keto-L-gulonic acid (2-KLG) using NADH as a cofactor.  2-KLG is a
     valuable precursor to ascorbic acid, or vitamin C, and an enzyme with
     increased activity with NADH may be able to improve two potential vitamin
     C prodn. processes.  Previously we have identified three amino acid
     residues that can be mutated to improve activity with NADH as a cofactor.
     As a pilot study to show feasibility, a library was made with these three
```

amino acids randomized, and 300 random colonies were screened for increased NADH activity. The activities of seven mutants with apparent improvements were verified using activity-stained native gels, and sequencing showed that the amino acids obtained were similar to some of those already discovered using rational design. The four most active mutants were purified and kinetically characterized. All of the new mutations resulted in apparent kcat values that were equal to or higher than that of the best mutant obtained through rational design. At satg. levels of cofactor, the best mutant obtained was almost twice as active with NADH as a cofactor as the wild-type enzyme is with NADPH. This screen is a valuable tool for improving ***2*** , ***5*** - ***DKG*** reductase, and it could easily be modified for improving other aspects of this protein or similar enzymes.

L12 ANSWER 3 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

2002:312255 Document No. 137:59418 Optimizing an Artificial Metabolic Pathway: Engineering the Cofactor Specificity of Corynebacterium 2,5-Diketo-D-gluconic Acid Reductase for Use in Vitamin C Biosynthesis. Banta, Scott; Swanson, Barbara A.; Wu, Shan; Jarnagin, Alisha; Anderson, Stephen (Departments of Chemical and Biochemical Engineering and of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ, 08854, USA). Biochemistry, 41(20), 6226-6236 (English) 2002. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB The strict cofactor specificity of many enzymes can potentially become a liability when these enzymes are to be employed in an artificial metabolic pathway. The preference for NADPH over NADH exhibited by the Corynebacterium 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase may not be ideal for use in industrial scale vitamin C biosynthesis. We have previously reported making a no. of site-directed mutations at five sites located in the cofactor-binding pocket that interact with the 2'-phosphate group of NADPH. These mutations conferred greater activity with NADH upon the Corynebacterium ***2*** , ***5*** - ***DKG*** reductase [Banta, S., Swanson, B. A., Wu, S., Jarnagin, A., and Anderson, S. (2002) Protein Eng. 15, 131-140;]. The best of these mutations have now been combined to see if further improvements can be obtained. In addn., several chimeric mutants have been produced that contain the same residues as are found in other members of the aldo-keto reductase superfamily that are naturally able to use NADH as a cofactor. The most active mutants obtained in this work were also combined with a previously reported substrate-binding pocket double mutant, F22Y/A272G. Mutant activity was assayed using activity-stained native polyacrylamide gels. Superior mutants were purified and subjected to a simplified kinetic anal. The simplified kinetic anal. was extended for the most active mutants in order to obtain the kinetic parameters in the full-ordered bi bi rate equation in the absence of products, with both NADH and NADPH as cofactors. The best mutant ***2*** , ***5*** - ***DKG*** reductase produced in this work was the F22Y/K232G/R238H/A272G quadruple mutant, which exhibits activity with NADH that is more than 2 orders of magnitude higher than that of the wild-type enzyme, and it retains a high level of activity with NADPH. This new ***2*** , ***5*** - ***DKG*** reductase may be a valuable new catalyst for use in vitamin C biosynthesis.

L12 ANSWER 4 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

2002:296628 Document No. 137:59456 Alteration of the specificity of the cofactor-binding pocket of Corynebacterium 2,5-diketo-D-gluconic acid reductase A. Banta, Scott; Swanson, Barbara A.; Wu, Shan; Jarnagin, Alisha; Anderson, Stephen (Departments of Chemical and Biochemical Engineering, Rutgers, The State University of New Jersey, Piscataway, NJ, 08854, USA). Protein Engineering, 15(2), 131-140 (English) 2002. CODEN: PRENE9. ISSN: 0269-2139. Publisher: Oxford University Press.

AB The NADPH-dependent 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase enzyme is a required component in some novel biosynthetic vitamin C prodn. processes. This enzyme catalyzes the conversion of ***2*** , ***5*** - ***DKG*** to 2-keto-L-gulonic acid, which is an immediate precursor to L-ascorbic acid. Forty unique site-directed mutations were made at five residues in the cofactor-binding pocket of ***2*** , ***5*** - ***DKG*** reductase A to improve its ability to use NADH as a cofactor. NADH is more stable, less expensive and more prevalent in the cell than is NADPH. To the best of the authors'

knowledge, this is the first focused attempt to alter the cofactor specificity of a member of the aldo-keto reductase superfamily by engineering improved activity with NADH into the enzyme. Activity of the mutants with NADH or NADPH was assayed using activity-stained native polyacrylamide gels. Eight of the mutants at three different sites were identified as having improved activity with NADH. These mutants were purified and subjected to a kinetic characterization with NADH as a cofactor. The best mutant obtained, R238H, produced an almost 7-fold improvement in catalysis with NADH compared with the wild-type enzyme. Surprisingly, most of this catalytic improvement appeared to be due to an improvement in the apparent kcat for the reaction rather than a large improvement in the affinity of the enzyme for NADH.

L12 ANSWER 5 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2002:123238 Document No. 136:182550 Enhanced 2-keto-L-gulonic acid production. Kumar, Manoj; Valle, Fernando; Dartois, Veronique A.; Hoch, James A. (Genencor International, Inc., USA; Microgenomics, Inc.). PCT Int. Appl. WO 2002012528 A2 20020214, 56 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24327 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.
AB A method for enhancing a host cell's biosynthetic prodn. 2-keto-L-gulonic acid (2-KLG) of is described. Such method comprises selecting a host cell that has an at least partially intracellular synthetic pathway which utilizes 2,5-diketogluconic acid (***2*** , ***5*** - ***DKG***) to produce 2-keto-L-gulonic acid; increasing the transport of ***2*** , ***5*** - ***DKG*** into cell while maintaining the integrity of the cell; culturing the cell to produce ***2*** , ***5*** - ***DKG*** ; and producing 2-KLG. The transport of the ***2*** , ***5*** - ***DKG*** is increased by transforming into the host cell DNA encoding for one or more transport protein to enhance the supply of the ***2*** , ***5*** - ***DKG*** into the host cell.

L12 ANSWER 6 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2002:123204 Document No. 136:166157 Increasing industrial production of metabolites by increasing levels of substrate uptake by cells by expression of foreign genes for transport proteins. Kumar, Manoj; Valle, Fernando (Genencor International, Inc., USA). PCT Int. Appl. WO 2002012481 A2 20020214, 60 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24600 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.
AB A method for improving yields of a metabolite by a cell by increasing the efficiency of uptake of a precursor of the metabolite is described. The transport of the substrate is increased by transforming into the host cell DNA encoding for one or more enzymes transporting the substrate into the host cell. The invention provides protein and cDNA sequences of novel proteins from Klebsiella, Pantoea having 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) permease activity. The isolated nucleic acid mols. can be expressed in appropriate bacterial cells to enhance the prodn. of 2-keto-L-Gluconic acid (2-KLG), which can subsequently be converted to ascorbic acid. Further provided are isolated polypeptides having ***2*** , ***5*** - ***DKG*** permease activity, immunogenic peptides therefrom, and antibodies specific therefor. The invention also provides methods of identifying novel ***2*** , ***5*** - ***DKG*** permeases.

L12 ANSWER 7 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2002:123191 Document No. 136:179592 Protein and cDNA sequences of

2,5-diketo-D-gluconic acid transport proteins and gluconate reductases from Klebsiella, Pantoea and other unknown microorganisms. Dartois, Veronique A.; Hoch, James A.; Valle, Fernando; Kumar, Manoj (Microgenomics, Inc., USA; Genencor International, Inc.). PCT Int. Appl. WO 2002012468 A2 20020214, 90 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24507 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.

AB The invention provides protein and cDNA sequences of novel proteins from Klebsiella, Pantoea and other unknown microorganisms, having 2,5-Diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) permease, 2-keto-reductase or 5-keto-reductase activity. The isolated nucleic acid mols. can be expressed in appropriate bacterial cells to enhance the prodn. of 2-keto-L-Gluconic acid (2-KLG), which can subsequently be converted to ascorbic acid. Further provided are isolated polypeptides having ***2*** , ***5*** - ***DKG*** permease activity, immunogenic peptides therefrom, and antibodies specific therefor. The invention also provides methods of identifying novel ***2*** , ***5*** - ***DKG*** permeases.

L12 ANSWER 8 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

2001:658849 Document No. 135:343351 Substrate selectivity of Gluconobacter oxydans for production of 2,5-diketo-D-gluconic acid and synthesis of 2-keto-L-gulonic acid in a multienzyme system. Ji, Aiguo; Gao, Peiji (Department of Pharmacy, Shandong University, Jinan, 250012, Peop. Rep. China). Applied Biochemistry and Biotechnology, 94(3), 213-223 (English) 2001. CODEN: ABIBDL. ISSN: 0273-2289. Publisher: Humana Press Inc..

AB Substrate selectivity of G. oxydans (ATCC 9937) for 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) prodn. was investigated with glucose, gluconic acid, and gluconolactone in different concns. using a resting-cell system. The results show that gluconic acid was utilized favorably by G. oxydans as substrate to produce ***2*** , ***5*** - ***DKG*** . The strain was coupled with glucose dehydrogenase (GDH) and ***2*** , ***5*** - ***DKG*** reductase for synthesis of 2-keto-L-gulonic acid (2-KLG), a direct precursor of L-ascorbic acid, from glucose. NADP and NADPH were regenerated between GDH and ***2*** , ***5*** - ***DKG*** reductase. The mole yield of 2-KLG of this multienzyme system was 16.8%. There are 3 advantages for using the resting cells of G. oxydans to connect GDH with ***2*** , ***5*** - ***DKG*** reductase for prodn. of 2-KLG: gluconate produced by GDH may immediately be transformed into ***2*** , ***5*** - ***DKG*** so that a series of problems generally caused by the accumulation of gluconate would be avoided; ***2*** , ***5*** - ***DKG*** is supplied directly and continuously for ***2*** , ***5*** - ***DKG*** reductase, so it is unnecessary to take special measures to deal with this unstable substrate as it was in Sonoyama's tandem fermn. process; and NADP(H) was regenerated within the system without any other components or systems.

L12 ANSWER 9 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

2001:420343 Document No. 135:192107 Structural Assembly of the Active Site in an Aldo-keto Reductase by NADPH Cofactor. Sanli, Gulsah; Blaber, Michael (Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL, 32306-4380, USA). Journal of Molecular Biology, 309(5), 1209-1218 (English) 2001. CODEN: JMOBAK. ISSN: 0022-2836. Publisher: Academic Press.

AB A 1.9 .ANG. resoln. x-ray structure of the apo-form of Corynebacterium 2,5-diketo-D-gluconic acid reductase A (2,5-DKGR A), a member of the aldo-keto reductase superfamily, has been detd. by mol. replacement using the NADPH-bound form of the same enzyme as the search model. 2,5-DKGR A catalyzes the NADPH-dependent stereo-specific redn. of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) to 2-keto-L-gluconate, a precursor in the industrial prodn. of vitamin C. An at.-resoln. structure for the apo-form of the enzyme, in conjunction with our previously reported high-resoln. x-ray structure for the holo-enzyme

and holo/substrate model, allows a comparative anal. of structural changes that accompany cofactor binding. The results show that regions of the active site undergo coordinated conformational changes of up to 8 .ANG.. These conformational changes result in the organization and structural rearrangement of residues assocd. with substrate binding and catalysis. Thus, NADPH functions not only to provide a hydride ion for catalytic redn., but is also a crit. structural component for formation of a catalytically competent form of DKGR A. (c) 2001 Academic Press.

L12 ANSWER 10 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2001:398476 Document No. 135:60217 Analysis of microbial growth of Erwinia citreus in continuous culture. Zelic, B.; Pavlovic, N.; Vasic-Racki, D. (Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, 10000, Croatia). Recents Progres en Genie des Procedes, 13(71), 303-310 (English) 1999. CODEN: RPGPEX. ISSN: 1166-7478. Publisher: Tec & Doc - Lavoisier.
AB Dynamic responses of the continuous culture of Erwinia to the pulse of gluconic acid (GA), 2-keto-D-gluconic acid (2-KDG), and 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) are described. Correlations of these responses with the biomass growth rate are found. The model parameters were detd. from the kinetic anal. of the exptl. data. The developed model is compared with the exptl. data of the growth of Erwinia sp. A reasonable agreement was obsd. between the model simulations and the exptl. data for the continuous culture growth on glucose, GA, 2-KDG and ***2*** , ***5*** - ***DKG*** , as well as for the batch fermn.

L12 ANSWER 11 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2001:380080 Document No. 135:121243 2,5-Diketo-D-gluconate production by a mixed culture of two newly-isolated strains: Flavimonas oryzihabitans and Pseudomonas cepacia. Sulo, Pavol; Hudecova, Daniela; Properova, Antonia; Basnak, Ivan; Sedlacek, Ivo (Institute of Biotechnology, Slovak Technical University, Bratislava, 82137, Slovakia). Biotechnology Letters, 23(9), 693-696 (English) 2001. CODEN: BILED3. ISSN: 0141-5492. Publisher: Kluwer Academic Publishers.
AB A mixed culture of two Gram-neg. bacteria isolated from soil converted 50 g D-glucose l-1 to 2,5-diketo-D-gluconate (***2*** , ***5*** ***DKG***) in 92% yield within 150 h. The first strain, producing 2-keto-D-gluconate (2 KDG) from D-glucose via D-gluconate (DG), was classified as Flavimonas oryzihabitans. The second strain, that converts 2 KDG to ***2*** , ***5*** ***DKG*** , was identified as Pseudomonas cepacia. This approach presents a new possibility to produce ascorbic acid by microbial transformation, including the use of other, more convenient substrates.

L12 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:905098 Document No. 134:233426 Preparation of NAD-Sepharose 6MB and its application in purification of 2,5-diketo-D-gluconic acid reductase. Ji, Aiguo; Gao, Peiji (Dept. of Biopharmaceutics, Shandong Medical University, Jinan, 250012, Peop. Rep. China). Shandong Yike Daxue Xuebao, 38(3), 258-259 (Chinese) 2000. CODEN: SYXBEE. ISSN: 1000-0496. Publisher: Shandong Yike Daxue.
AB NAD-Sepharose 6MB affinity gel was prepd. and its utility in sepn. and purifn. of 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase was studied. NAD was combined with Sepharose 6MB by succinate acyl hydrazides to get NAD-Sepharose 6MB affinity gel. Crude ***2*** , ***5*** - ***DKG*** reductase was sepd. from Corynebacterium sp.ATCC 31090. The prepd. NAD affinity gel was used for purifn. of ***2*** , ***5*** - ***DKG*** reductase from the crude enzyme. The combination rate of NAD to Sepharose 6MB was 71.67%. The recovery of affinity chromatog. was 77.27%. The specific activity of the enzyme after purifn. was 1.1 times higher than before. The NAD-Sepharose 6MB affinity gel is effective for purifn. of ***2*** , ***5*** - ***DKG*** reductase.

L12 ANSWER 13 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:854605 Document No. 134:161912 Manufacture of 2-keto-L-gluconic acid from D-glucose by one-step fermentation. Chen, Ce-shi; Yin, Guang-lin (Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China). Shengwu Gongcheng Jinzhan, 20(5), 51-56 (Chinese) 2000. CODEN: SGJHA2. ISSN: 1003-3505. Publisher: Zhongguo Kexueyuan Wenxian Qingbao Zhongxin.

AB A review with with 20 refs. on the research progress of producing 2-keto-L-gluconic acid, an important precursor of L-ascorbic acid, from D-glucose using recombinant microorganisms. The background of producing 2-KLG from D-glucose by tandem-fermn. and one step fermn. through gene engineering techniques is introduced. Then,techniques to optimize the expression of ***2*** , ***5*** - ***DKG*** reductase and block the side-pathways by creating mutants in the 2-keto aldose reductase genes in vitro and in vivo by allelic replacement is focused. At the same time, the protein engineering of ***2*** , ***5*** - ***DKG*** reductase is also mentioned. Finally, the future of this research and possibility of its application are discussed.

L12 ANSWER 14 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:476011 Document No. 133:70701 Preparation of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) reductase in Escherichia coli and Erwinia. Yin, Guanglin; Chen, Ceshi (Shanghai Research Center of Biological Engineering, Chinese Academy of Sciences, Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1221792 A 19990707, 10 pp. (Chinese). CODEN: CNXXEV. APPLICATION: CN 1997-125210 19971230.
AB The present invention relates to the prepn. of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) reductase which can be applied to improve prodn. efficiency of 2,5-diketo-gluconic acid (2-KLG, vitamin C precursor) from glucose. The ***2*** , ***5*** - ***DKG*** reductase gene is cloned from Corynebacterium sp. strain SCB3058 and mutated by PCR to change nucleotide A434.fwdarw.G and nucleotide G734.fwdarw.C for His145.fwdarw.Arg and Val245.fwdarw.Ala substitution. The modified ***2*** , ***5*** - ***DKG*** gene is placed under the control of Plac (lactase gene promoter) in the plasmid PBL4 and the distance between translation initiation signal SD and AUG codon is 8bp. Streptomycin-resistance gene is used as the selection marker. Plasmid PBL4 (Escherichia coli vector) is modified to plasmid PBLS for gene expression in Erwinia.

L12 ANSWER 15 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:249398 Document No. 133:236885 Production of 2-KLG from glucose by constructing recombinant bacteria. Qiao, Chunhong; Chen, Ceshi; Chen, Fang; Li, Yue; Yin, Guanglin (Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China). Gongye Weishengwu, 30(1), 9-14 (Chinese) 2000. CODEN: GOWEEK. ISSN: 1001-6678. Publisher: Quanguo Gongye Weishengwu Xinxi Zhongxin.
AB Two recombinant E. coli BL21(DE3) PET9aII and DH5.alpha. (pBL4) and one recombinant Erwinia capable of expressing 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase, were constructed after 2 genes encoding them were cloned from Corynebacterium SCB3058. The expression of ***2*** , ***5*** - ***DKG*** reductase was controlled by PL or T7 promoter. The enzyme activity was induced by raising temp. or adding IPTG. It was also confirmed by SDS-PAGE. These recombinant E. coli and Erwinia were used to transform ***2*** , ***5*** - ***DKG*** in vitro and in vivo. All these recombinant bacteria could produce 2-keto-L-gulonic acid (2-KLG) in vitro and in vive. Both the media formula and style of inducement were very important for transformation in flask. The yields might be related to NADPH concn., 2-KLG transport out from the cell and the 2-KLG reductive enzyme.

L12 ANSWER 16 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:235380 Document No. 133:2315 Genetic and biochemical characterization of the pathway in Pantoea citrea leading to pink disease of pineapple. Pujol, Catherine J.; Kado, Clarence I. (Department of Plant Pathology, University of California, Davis, CA, 95616, USA). Journal of Bacteriology, 182(8), 2230-2237 (English) 2000. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.
AB Pink disease of pineapple, caused by Pantoea citrea, is characterized by a dark coloration on fruit slices after autoclaving. This coloration is initiated by the oxidn. of glucose to gluconate, which is followed by further oxidn. of gluconate to as yet unknown chromogenic compds. To elucidate the biochem. pathway leading to pink disease, we generated six coloration-defective mutants of P. citrea that were still able to oxidize glucose into gluconate. Three mutants were found to be affected in genes involved in the biogenesis of c-type cytochromes, which are known for their role as specific electron acceptors linked to dehydrogenase activities. Three addnl. mutants were affected in different genes within

an operon that probably encodes a 2-ketogluconate dehydrogenase protein. These six mutants were found to be unable to oxidize gluconate or 2-ketogluconate, resulting in an inability to produce the compd. 2,5-diketogluconate (***2*** , ***5*** - ***DKG***). Thus, the prodn. of ***2*** , ***5*** - ***DKG*** by P. citrea appears to be responsible for the dark color characteristic of the pink disease of pineapple.

L12 ANSWER 17 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
2000:145741 Document No. 132:319197 Molecular modeling of substrate binding in wild-type and mutant Corynebacteria 2,5-diketo-D-gluconate reductases. Khurana, Sumit; Sanli, Gulsah; Powers, David B.; Anderson, Stephen; Blaber, Michael (Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL, 32306-3015, USA). Proteins: Structure, Function, and Genetics, 39(1), 68-75 (English) 2000. CODEN: PSFGEY. ISSN: 0887-3585. Publisher: Wiley-Liss, Inc..
AB 2,5-Diketo-D-gluconic acid reductase (2,5-DKGR; E.C. 1.1.1.-) catalyzes the NADP (NADPH)-dependent stereo-specific redn. of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) to 2-keto-L-gulonate (2-KLG), a precursor in the industrial prodn. of vitamin C (L-ascorbate). Microorganisms that naturally ferment D-glucose to ***2*** , ***5*** - ***DKG*** can be genetically modified to express the gene for 2,5-DKGR, and thus directly produce vitamin C from D-glucose. Two naturally occurring variants of DKGR (DKGR A and DKGR B) have been reported. DKGR B exhibits higher specific activity toward ***2*** , ***5*** - ***DKG*** than DKGR A; however, DKGR A exhibits a greater selectivity for this substrate and significantly higher thermal stability. Thus, a modified form of DKGR, combining desirable properties from both enzymes, would be of substantial com. interest. In the present study we use a mol. dynamics-based approach to understand the conformational changes in DKGR A as the active site is mutated to include two active site residue changes that occur in the B form. The results indicate that the enhanced kinetic properties of the B form are due, in part, to residue substitutions in the binding pocket. These substitutions augment interactions with the substrate or alter the alignment with respect to the putative proton donor group.

L12 ANSWER 18 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1999:790388 Document No. 132:330427 Cloning and expression of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) reductase gene in Escherichia coli.. Li, Yue; Chen, Ceshi; Yin, Guanglin (Shanghai Research Center of Biotechnology, Chinese Academy of Sciences, Shanghai, 200233, Peop. Rep. China). Weishengwuxue Tongbao, 26(4), 260-265 (Chinese) 1999. CODEN: WSWPDI. ISSN: 0253-2654. Publisher: Kexue Chubanshe.
AB PCR method was used to amplify the gene of 2,5-diketo-D- gluconate reductase II with the template of chromosomal DNA from Corynebacterium sp. strain SCB3058. Restriction enzyme sequence FdcoR I and BamH I were added to the end of ***2*** , ***5*** - ***DKG*** reductase gene resp. by PCR techniques. The gene was cloned with a cloning vector pGEM-T and expressed with an expression vector pBV220. No exact protein was obsd. after induced at 42.degree.. Sequence detn. showed that one base was lost in PCR primer which led to the loss of end code, also three bases before ATG combined to other three bases in SD sequences which affected the expression. The right ***2*** , ***5*** - ***DKG*** reductase gene was got and expressed in vector pBV220 pBL4 successfully.

L12 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1999:442188 Document No. 131:270988 Conversion properties of Gluconobacter oxydans to different substrates and 2-keto-L-gulonic acid synthesis by Co-Immobilized cells of G. Oxydans and Corynebacterium sp.. Ji, Aiguo; Gao, Peiji (Shandong Medical University, Jinan, 250012, Peop. Rep. China). Yaowu Shengwu Jishu, 6(1), 14-19 (Chinese) 1999. CODEN: YSJIFO. ISSN: 1005-8915. Publisher: Yaowu Shengwu Jishu Bianjibu.
AB The metabolic properties of free and immobilized cells of Gluconobacter oxydans (ATCC 9937) for 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) prodn. were investigated with glucose (G), gluconic acid(GA) and G, GA mixt. as substrates in different concns. The results showed that gluconic acid and gluconolactone were utilized prior to glucose by the cells of G. oxydans as substrate to produce ***2*** , ***5*** - ***DKG*** . The concn. of 5% was suitable for ***2*** , ***5*** -

. ***DKG*** prodn. to both free and immobilized cells of G. oxydans. The stability of immobilized cells of G. oxydans with polyvinylalc. (PVA)-alginate was improved. The prodn. of 2-keto-L-gulonic acid (2-KLG) from gluconic acid by co-immobilized cells of Gluconobacter oxydans (ATCC 9937) and Corynebacterium sp. (ATCC 31090) was investigated according to the 2,5-diketo-D-gluconate pathway of 2-KLG synthesis from glucose in bacteria. The cells of G. oxydans and Corynebacterium sp. were entrapped in different ratio with PVA-Alginate. Glucose, gluconic acid and the mixt. of glucose and gluconic acid were used as substrates, resp. When the ratio of two cells was 1:1 and the concn. of bacteria was 100 mg/mL, the yield of 2-KLG reached 37.72%.

L12 ANSWER 20 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1999:55287 Document No. 130:262789 Cloning and expression in Escherichia coli of 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase I from Corynebacterium. Che, Ceshi; Yin, Guanglin (Shinghai Res. Center Biotechnology, Chinese Academy Sciences, Shanghai, 200233, Peop. Rep. China). Weishengwu Xuebao, 38(6), 435-440 (Chinese) 1998. CODEN: WSHPA8. ISSN: 0001-6209. Publisher: Kexue Chubanshe.
AB Two different 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) reductase was purified from Corynebacterium species SCB3058, then its genomic DNA was used as template, a segment contg. ***2*** , ***5*** - ***DKG*** reductase I was amplified by PCR and cloned into pGEM3zf(+) to obtain the recombinant plasmid pGEM813. The sequence anal. showed that the cloned segment was 1107 bp in length, contained a single open reading frame of 834 nucleotides, which encoded a 34-kD protein consisted of 278 amino acids. After the primary control sequence was deleted, the expression vector pBL4 was constructed by plasmid pBL. With induction of temp., a 34-kD protein was specifically expressed in E. coli DH5.alpha.. The expressed recombinant protein accounted for 20% of the total cell protein and had high specific enzyme activity. In spite of 30.degree.C, 37.degree.C or 42.degree.C induction, the specific activity of enzyme was almost the same. Most of the recombinant protein induced at 42.degree.C existed with inclusion bodies. This result may be useful for constructing a recombinant Erwinia which can produce 2-keto-L-gulonic acid directly from D-glucose by one-step fermn.

L12 ANSWER 21 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1998:564164 Document No. 129:172451 Mutants of 2,5-diketo-D-gluconic acid reductase A for manufacture of vitamin C precursor 2-keto-L-gulonic acid. Powers, David B.; Anderson, Stephen (Rutgers, the State University of New Jersey, USA). U.S. US 5795761 A 19980818, 39 pp., Cont.-in-part of U. S. Ser. No. 584,019, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1996-585595 19960116. PRIORITY: US 1996-584019 19960111.
AB Mutants of 2,5-diketo-D-gluconic acid reductase A (***2*** , ***5*** - ***DKG***), an enzyme used to produce 2-keto-L-gulonic acid, a precursor of ascorbic acid (vitamin C), are prepd. by site-directed mutagenesis. These mutants may exhibit one or more of the following characteristics; improved temp. stability, increased resistance to substrate inhibition, increased turnover of the substrate by the enzyme and increased affinity for the substrate. The Q192R mutant of Corynebacterium ATCC 31090 ***2*** , ***5*** - ***DKG*** was prepd. with recombinant Acetobacter cerinus. The Vmax for ***2*** , ***5*** - ***DKG*** was improved 1.8-fold and the specificity const. 2.4-fold over the wild-type enzyme. Mutants with decreased sensitivity to substrate inhibition were also prepd.

L12 ANSWER 22 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1998:378477 Document No. 129:133009 Crystal structure of 2,5-diketo-D-gluconic acid reductase A complexed with NADPH at 2.1-.ANG. resolution. Khurana, Sumit; Powers, David B.; Anderson, Stephen; Blaber, Michael (Institute of Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, FL, 32306-3015, USA). Proceedings of the National Academy of Sciences of the United States of America, 95(12), 6768-6773 (English) 1998. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB The 3-dimensional crystal structure of Corynebacterium 2,5-diketo-D-gluconate reductase A (I), in complex with the cofactor, NADPH, was solved by using x-ray crystallog. data to 2.1-.ANG. resoln. I catalyzes the stereospecific redn. of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) to 2-keto-L-gulonate. Thus, the 3-dimensional

structure has now been solved for a prokaryotic example of the aldo-keto reductase superfamily. The details of the binding of NADPH helped explain why I exhibited lower binding affinity for cofactor than the related human aldose reductase does. Furthermore, changes in the local loop structure near the cofactor suggested that I will not exhibit the biphasic cofactor binding characteristics obsd. in aldose reductase. Although the crystal structure did not include the substrate, the 2 ordered water mols. present within the substrate-binding pocket were postulated to provide positional landmarks for the substrate 5-keto and 4-OH groups. The structural basis for several previously described active site mutants of I was also proposed. Recent research efforts have described a novel approach to the synthesis of L-ascorbate (vitamin C) by using a genetically engineered microorganism that is capable of synthesizing ***2*** , ***5*** - ***DKG*** from glucose and subsequently is transformed with the gene for I. These modifications create a microorganism capable of direct prodn. of 2-keto-L-gulonate from D-glucose, and the gulonate can subsequently be converted into vitamin C. In economic terms, vitamin C is the single most important specialty chem. manufd. in the world. Understanding the structural determinants of specificity, catalysis, and stability of I is thus of substantial com. interest.

L12 ANSWER 23 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1997:106572 Document No. 126:130634 Production of 2,5-diketogluconic acid in immobilized cell reactors.. Shin, Bong-Soo; Shin, Chul-Soo (Department of Food and Biotechnology, College of engineering and Bioproducts Research Center, Yonsei University, Seoul, 120-749, S. Korea). Sanop Misaengmul Hakhoechi, 24(6), 705-711 (Korean) 1996. CODEN: SMHAEH. ISSN: 0257-2389. Publisher: Korean Society for Applied Microbiology.
AB For the efficient prodn. of 2,5-diketogluconic acid (2,5-KDG) by the immobilized cells of Erwinia herbicola, basic characteristics of ***2*** , ***5*** - ***DKG*** fermn. were analyzed and a process employing immobilized cell reactor was developed. The immobilized cells appeared to have diffusion limitation, and a max. prodn. of ***2*** , ***5*** - ***DKG*** was accomplished with 2 mm diams. of immobilized beads. Long-term stabilities of the immobilized cells could be maintained by addn. of 1.75% (w/v) polypeptone. Repeted batch fermns. with about 80 mol% of ***2*** , ***5*** - ***DKG*** yields were carried out six times in the fluidized bubble column reactors filled with immobilized cells at an aeration rate of 6 vvm.

L12 ANSWER 24 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1996:582762 Document No. 125:296485 Determination of 2-keto-l-gulonic, 2-keto-d-gluconic and 2,5-diketo-d-gluconic acids by capillary zone electrophoresis. Choi, One-Kyun; Kim, Chang-Gon; Kim, Yong-Duk; Kim, Hun; Jo, Jae-Sun (Bioanalytical Laboratory, Dongyoung Instrumental, C.P.O. Box 7162, Seoul, S. Korea). Journal of Chromatography, A, 745(1+2), 249-254 (English) 1996. CODEN: JCRAEY. ISSN: 0021-9673. Publisher: Elsevier.
AB During the biosynthetic processing of 2-keto-l-gulonic acid (2-KLG) from 2,5-diketo-d-gluconic acid (***2*** , ***5*** - ***DKG***) by Corynebacterium sp., 2-keto-d-gluconic acid (2-KDG) is produced as a byproduct. These org. acids have been analyzed by HPLC on an Aminex HPX-87H column, but the resoln. was not good enough for quant. anal. We investigated the quantitation of 2-KLG, 2-KDG and ***2*** , ***5*** - ***DKG*** using capillary electrophoresis (CE) and the results were compared with those of HPLC. With CE, in contrast to HPLC, good resoln., efficiency and rapid anal. were demonstrated as well as low consumption of solvent and samples. The CE system was applied at 15 kV with UV detection at 195 nm using 100 mM sodium borate (pH 8.4) as an electrolyte. The results were shown within 5 min with efficiency approaching 100000 theor. plates. The relative std. deviations of migration time and peak area were less than 0.9% and 1.6%, resp. The detection limits for quant. detn. were 0.5-1.3 .mu.M level. The above compds., in fermn. broth, were analyzed under the optimum conditions. Considering the results of our study, the CE method should be highly suitable for the sepn. of 2-KLG, 2-KDG and ***2*** , ***5*** - ***DKG*** in the fermn. broth.

L12 ANSWER 25 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1992:632101 Document No. 117:232101 The technological conditions of producing 2-keto-L-gulonic acid from D-glucose by direct fermentation. Zhang, Gang; Lan, Xiande; Fan, Xiaobing; Han, Zhihua; Yin, Guanglin; Ma, Zhifang; Dong, Wenling (Biol. Sci. Technol. Dep., Shanghai Jiaolong Univ.,

Shanghai, 200030, Peop. Rep. China). Weishengwuxue Tongbao, 19(2), 78-81 (Chinese) 1992. CODEN: WSWPDI. ISSN: 0253-2654.

AB 2-Keto-L-gulonic acid (2-KLG), the precursor of L-ascorbic acid synthesis, was prepd. directly from D-glucose by tandem fermn. In the 1st step, Erwinia sp. SCB 247 transformed D-glucose to 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***), which accumulated 180 mg ***2*** , ***5*** - ***DKG*** /mL. In the 2nd step, Corynebacterium sp. SCB 3058 reduced ***2*** , ***5*** - ***DKG*** to 2-KLG, accumulating 35 mg 2-KLG/mL. This reductive fermn. was obtained under aerobic conditions by adding a H donor such as glucose. The av. yield of 5 batch fermns. was 56.3 mol% 2-KLG from D-glucose in a 10-L fermentor.

L12 ANSWER 26 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

1992:169304 Document No. 116:169304 Determination of keto-acids in bioconversion of D-glucose by bacterial genera of Erwinia and Gluconobacter by thin-layer chromatography. Joveva, Suzana; Gamulin, S.; Golubic, Z.; Nozinic, Ranka; Sunic, D.; Delic, V. (Istraz. Inst., PLIVA, Zagreb, Yugoslavia). Prehrambeno-Tehnoloska i Biotehnoloska Revija, 29(1), 25-9 (Serbo-Croatian) 1991. CODEN: PTBREK. ISSN: 0352-9193.

AB TLC was used for qual. and quant. detn. of intermediates accumulated as a result of glucose bioconversion by Gluconobacter and Erwinia. Bacteria were cultivated in liq. glucose medium and keto-acids as intermediates were detd. on silica gel G and silica gel G plates impregnated with 5 percent meta-phosphoric acid with different combinations of solvents. A good sepn. of D-gluconic, 2-keto and 5-keto-D-gluconic, 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) and D-glucose was achieved. After development and spots elution, intensity of colors was measured by spectroscopy and the results were evaluated statistically. Results obtained by TLC for D-glucose and ***2*** , ***5*** - ***DKG*** were compared by colorimetric methods on an automated analyzer. A good correlation of these results was obtained.

L12 ANSWER 27 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

1992:57516 Document No. 116:57516 Metabolic pathway engineering to increase production of ascorbic acid intermediate 2-keto-L-gulonic acid. Anderson, Stephen; Lazarus, Robert A.; Miller, Harvey I.; Stafford, R. Kevin (Genentech, Inc., USA). U.S. US 5032514 A 19910716, 34 pp. (English). CODEN: USXXAM. APPLICATION: US 1988-229598 19880808.

AB Improvement is made in the process for converting glucose into 2-keto-L-gulonic acid (2-KLG), a stable storable precursor for ascorbic acid. In recombinant microorganisms which were rendered capable of converting 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) to 2-KLG by transfer of genetic material, the secondary metabolites and metabolic pathways leading to the metabolic diversion of 2-KLG and ***2*** , ***5*** - ***DKG*** were detd., and the diversion of 2-KLG to L-iodonic acid (IA) or of ***2*** , ***5*** - ***DKG*** to 5-keto-D-gluconate (5-KDH) was blocked. Erwinia herbicola was transformed with plasmid p269, an expression vector for ***2*** , ***5*** - ***DKG*** reductase. In addn. to prodn. of desired metabolites, IA was produced. 2-Ketoaldonate reductase (2-KR), catalyzing the stereospecific redn. of 2-KLG to IA, was identified, purified and characterized. The gene encoding 2-KR(A), tkrA, was cloned and deleted from the E. herbicola chromosome by deletion mutagenesis. However, IA was still produced in the tkrA- mutant and a second 2-KR, 2-KR(B) was discovered. The DNA and amino acid sequences of 2-KR(A) and 2-KR(B) are shown.

L12 ANSWER 28 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN

1991:675494 Document No. 115:275494 Pathways for metabolism of ketoaldonic acids in an Erwinia sp. Truesdell, Susan J.; Sims, Jeffrey C.; Boerman, Patrice A.; Seymour, Jana L.; Lazarus, Robert A. (Pfizer Cent. Res., Groton, CT, 06340, USA). Journal of Bacteriology, 173(21), 6651-6 (English) 1991. CODEN: JOBAAY. ISSN: 0021-9193.

AB The pathways involved in the metab. of ketoaldonic acids by Erwinia sp. strain ATCC 39140 have been investigated by use of a combination of enzyme assays and isolation of bacterial mutants. The catabolism of 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) to gluconate can proceed by two sep. NAD(P)H-dependent pathways. The first pathway involves the direct redn. of ***2*** , ***5*** - ***DKG*** to 5-keto-D-gluconate, which is then reduced to gluconate. The second pathway involves the consecutive redn. of ***2*** , ***5*** - ***DKG*** to 2-keto-L-gulonate and L-idonic acid, which is then oxidized

to 5-keto-D-gluconate, which is then reduced to gluconate. Gluconate, which can also be produced by the NAD(P)H-dependent redn. of 2-keto-D-gluconate, is phosphorylated to 6-phosphogluconate and further metabolized through the pentose phosphate pathway. No evidence was found for the existence of the Entner-Doudoroff pathway in this strain.

L12 ANSWER 29 OF 29 CAPLUS COPYRIGHT 2003 ACS on STN
1987:528477 Document No. 107:128477 Production of a vitamin C precursor using genetically modified organisms. Hardy, Kimber; Van de Pol, Hendrick; Grindley, June; Payton, Mark A. (Biogen N. V., Neth.). PCT Int. Appl. WO 8700863 A1 19870212, 37 pp. DESIGNATED STATES: W: AU, DK, JP, US; RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1986-US1571 19860801. PRIORITY: GB 1985-19536 19850802; US 1985-792432 19851029.

AB Plasmids encoding the gene for 2,5-diketo-D-gluconate (DKG) reductase were transformed into Erwinia so that the transformant converts glucose or other C sources to the Vitamin C precursor 2-keto-L-gluconic acid (2-KLG) in a single fermn. process. The gene for 2,5-diketo-D-gluconate (***2*** , ***5*** - ***DKG***) reductase of Corynebacterium was cloned onto plasmid pCBR 13 and further cloned onto pPLred 332 under the control of .lambda. PL promoter. E. citreus Transformed with recombinant plasmid pPLred 332 was able to ferment 49.4% of glucose substrate to yield 19.83 g/L of 2-KLG.

```
=> S 2,5(W)DKG(W)PERMEASE
       7782983 2
       5454920 5
        599910 2,5
                (2(W)5)
           103 DKG
          2914 PERMEASE
           676 PERMEASES
          3148 PERMEASE
                (PERMEASE OR PERMEASES)
L13          2 2,5(W)DKG(W)PERMEASE

=> D 1-2 CBIB ABS
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L13 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
2002:123204 Document No. 136:166157 Increasing industrial production of metabolites by increasing levels of substrate uptake by cells by expression of foreign genes for transport proteins. Kumar, Manoj; Valle, Fernando (Genencor International, Inc., USA). PCT Int. Appl. WO 2002012481 A2 20020214, 60 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24600 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.

AB A method for improving yields of a metabolite by a cell by increasing the efficiency of uptake of a precursor of the metabolite is described. The transport of the substrate is increased by transforming into the host cell DNA encoding for one or more enzymes transporting the substrate into the host cell. The invention provides protein and cDNA sequences of novel proteins from Klebsiella, Pantoea having 2,5-diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) ***permease*** activity. The isolated nucleic acid mols. can be expressed in appropriate bacterial cells to enhance the prodn. of 2-keto-L-Gluconic acid (2-KLG), which can subsequently be converted to ascorbic acid. Further provided are isolated polypeptides having ***2*** , ***5*** - ***DKG*** ***permease*** activity, immunogenic peptides therefrom, and antibodies specific therefor. The invention also provides methods of identifying novel ***2*** , ***5*** - ***DKG*** ***permeases*** .

L13 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2003 ACS on STN
2002:123191 Document No. 136:179592 Protein and cDNA sequences of

2,5-diketo-D-gluconic acid transport proteins and gluconate reductases from Klebsiella, Pantoea and other unknown microorganisms. Dartois, Veronique A.; Hoch, James A.; Valle, Fernando; Kumar, Manoj (Microgenomics, Inc., USA; Genencor International, Inc.). PCT Int. Appl. WO 2002012468 A2 20020214, 90 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24507 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.

AB The invention provides protein and cDNA sequences of novel proteins from Klebsiella, Pantoea and other unknown microorganisms, having 2,5-Diketo-D-gluconic acid (***2*** , ***5*** - ***DKG***) ***permease*** , 2-keto-reductase or 5-keto-reductase activity. The isolated nucleic acid mols. can be expressed in appropriate bacterial cells to enhance the prodn. of 2-keto-L-Gluconic acid (2-KLG), which can subsequently be converted to ascorbic acid. Further provided are isolated polypeptides having ***2*** , ***5*** - ***DKG*** ***permease*** activity, immunogenic peptides therefrom, and antibodies specific therefor. The invention also provides methods of identifying novel ***2*** , ***5*** - ***DKG*** ***permeases*** .

```
=> S (DIKETO OR DI-KETO)(W)(D GLUCONIC ACID PERMEASE)
          2566 DIKETO
             2 DIKETOS
          2568 DIKETO
                  (DIKETO OR DIKETOS)
        427624 DI
          3962 DIS
        431431 DI
                  (DI OR DIS)
         51939 KETO
             6 KETOS
         51945 KETO
                  (KETO OR KETOS)
            41 DI-KETO
                  (DI(W)KETO)
       2004706 D
          8118 GLUCONIC
       3671955 ACID
       1390456 ACIDS
       4134012 ACID
                  (ACID OR ACIDS)
          2914 PERMEASE
           676 PERMEASES
          3148 PERMEASE
                  (PERMEASE OR PERMEASES)
             0 D GLUCONIC ACID PERMEASE
                  (D(W)GLUCONIC(W)ACID(W)PERMEASE)
L14           0 (DIKETO OR DI-KETO)(W)(D GLUCONIC ACID PERMEASE)

=> S (DIKETO OR DI-KETO)(W)(D GLUCONIC ACID)
          2566 DIKETO
             2 DIKETOS
          2568 DIKETO
                  (DIKETO OR DIKETOS)
        427624 DI
          3962 DIS
        431431 DI
                  (DI OR DIS)
         51939 KETO
             6 KETOS
         51945 KETO
                  (KETO OR KETOS)
            41 DI-KETO
                  (DI(W)KETO)
```

```
      2004706 D
         8118 GLUCONIC
      3671955 ACID
      1390456 ACIDS
      4134012 ACID
                (ACID OR ACIDS)
         1532 D GLUCONIC ACID
                (D(W)GLUCONIC(W)ACID)
L15        71 (DIKETO OR DI-KETO)(W)(D GLUCONIC ACID)

=> S PERMEASE
         2914 PERMEASE
          676 PERMEASES
L16      3148 PERMEASE
                (PERMEASE OR PERMEASES)

=> S L15 AND L16
L17         2 L15 AND L16

=> S L17 NOT L13
L18         0 L17 NOT L13

=> S PANTOEA;S CITREA;S L19(W)L20
L19       577 PANTOEA


L20        96 CITREA


L21        22 L19(W)L20

=> S L19 AND L16;S L21 AND L16
L22         3 L19 AND L16


L23         2 L21 AND L16

=> S L22,L23
L24         3 (L22 OR L23)

=> S L24 NOT L13
L25         1 L24 NOT L13

=> D CBIB ABS

L25  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2003 ACS on STN
1999:721573   Document No. 132:74326  Distribution of tetracycline resistance
     genes and transposons among phylloplane bacteria in Michigan apple
     orchards.  Schnabel, Elise L.; Jones, Alan L. (Department of Botany and
     Plant Pathology, Michigan State University, East Lansing, MI, 48824-1312,
     USA).  Applied and Environmental Microbiology, 65(11), 4898-4907 (English)
     1999.  CODEN: AEMIDF.  ISSN: 0099-2240.  Publisher: American Society for
     Microbiology.
AB   The extent and nature of tetracycline resistance in bacterial populations
     of two apple orchards with no or a limited history of oxytetracycline
     usage were assessed.  Tetracycline-resistant (Tcr) bacteria were mostly
     gram neg. and represented from 0 to 47% of the total bacterial population
     on blossoms and leaves (vs. 26 to 84% for streptomycin-resistant
     bacteria).. A total of 87 isolates were screened for the presence of
     specific Tcr determinants.  Tcr was detd. to be due to the presence of Tet
     B in   ***Pantoea***   agglomerans and other members of the family
     Enterobacteriaceae and Tet A, Tet C, or Tet G in most Pseudomonas
     isolates.  The cause of Tcr was not identified in 16% of the isolates
     studied.  The Tcr genes were almost always found on large plasmids which
     also carried the streptomycin resistance transposon Tn5393.  Transposable
     elements with Tcr determinants were detected by entrapment following
     introduction into Escherichia coli.  Tet B was found within Tn10.  Two of
     eighteen Tet B-contg. isolates had an insertion sequence within Tn10; one
     had IS911 located within IS10-R and one had Tn1000 located upstream of Tet
     B.  Tet A was found within a novel variant of Tn1721, named Tn1720, which
     lacks the left-end orfI of Tn1721.  Tet C was located within a 19-kb
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transposon, Tn1404, with transposition genes similar to those of Tn501, streptomycin (aadA2) and sulfonamide (sul1) resistance genes within an integron, Tet C flanked by direct repeats of IS26, and four open reading frames, one of which may encode a sulfate ***permease*** . Two variants of Tet G with 92% sequence identity were detected.

```
=> E DARTOIS V/AU
=> S E3-E5
             5 "DARTOIS V"/AU
            14 "DARTOIS VERONIQUE"/AU
             2 "DARTOIS VERONIQUE A"/AU
L26         21 ("DARTOIS V"/AU OR "DARTOIS VERONIQUE"/AU OR "DARTOIS VERONIQUE
               A"/AU)

=> E HOCH J/AU
=> S E3,E4,E13-E15
            26 "HOCH J"/AU
            30 "HOCH J A"/AU
             7 "HOCH JAMES"/AU
           321 "HOCH JAMES A"/AU
             1 "HOCH JAMES ALFRED"/AU
L27        381 ("HOCH J"/AU OR "HOCH J A"/AU OR "HOCH JAMES"/AU OR "HOCH JAMES
               A"/AU OR "HOCH JAMES ALFRED"/AU)

=> E VALLE F/AU
=> S E3,E12
            23 "VALLE F"/AU
            39 "VALLE FERNANDO"/AU
L28         62 ("VALLE F"/AU OR "VALLE FERNANDO"/AU)

=> E KUMAR M/AU
=> S E3,E30-E38,E161,E162
           265 "KUMAR M"/AU
             1 "KUMAR M K"/AU
             1 "KUMAR M K ANJAN"/AU
             1 "KUMAR M K HARI"/AU
             1 "KUMAR M K SURESH"/AU
             1 "KUMAR M KAMAL"/AU
             3 "KUMAR M KARUNA"/AU
             2 "KUMAR M KIRAN"/AU
             1 "KUMAR M KISHORE"/AU
             7 "KUMAR M KRISHNA"/AU
           139 "KUMAR MANOJ"/AU
             1 "KUMAR MANOJ K"/AU
L29        423 ("KUMAR M"/AU OR "KUMAR M K"/AU OR "KUMAR M K ANJAN"/AU OR "KUMA
               R M K HARI"/AU OR "KUMAR M K SURESH"/AU OR "KUMAR M KAMAL"/AU
               OR "KUMAR M KARUNA"/AU OR "KUMAR M KIRAN"/AU OR "KUMAR M KISHORE
               "/AU OR "KUMAR M KRISHNA"/AU OR "KUMAR MANOJ"/AU OR "KUMAR MANOJ
                K"/AU)

=> S L26,L27,L28,L29
L30        872 (L26 OR L27 OR L28 OR L29)

=> S L30 AND L19
L31          6 L30 AND L19

=> S L30 AND L16
L32          6 L30 AND L16

=> S L32,L31
L33         10 (L32 OR L31)

=> S L33 NOT (L13 OR L25)
L34          8 L33 NOT (L13 OR L25)

=> D 1-8 CBIB ABS

L34  ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2003 ACS on STN
2002:793750   Document No. 137:307296  Microorganisms with altered patterns of
     kinase activity that show altered balances between catabolic and
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biosynthetic metabolism. Dodge, Timothy C.; ***Valle, Fernando*** (Genencor International, Inc., USA). PCT Int. Appl. WO 2002081631 A2 20021017, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US10575 20020404. PRIORITY: US 2001-PV281618 20010404; US 2001-PV282259 20010405.

AB The invention provides methods for improving productivity of microbial producer cells in fermns. by altering the relationship between anabolism and catabolism. Specifically, enzymes affecting the phosphorylation of intermediates in carbohydrate and energy metab.: glucokinase, gluconokinase, phosphoenolpyruvate synthase and the HPr phosphotransferase system are modified to increase the yields of com. products. Such improved host cells are used for the prodn. of products, such as, ascorbic acid intermediates. Methods for making and using the improved host cells are provided. Nucleic acid and amino acid sequences for glucokinase and gluconokinase are provided. The glkA gene for the glucokinase of ***Pantoea*** citrea was cloned by complementation in Escherichia coli. The P. citrea glkA gene was then inactivated by homologous recombination. Similarly, the gntK gene for gluconokinase was cloned and inactivated. These cells were tested for the manner in which they utilized glucose or gluconic acid in the medium in metab. vs. ascorbic acid fermn. Wild-type cells passed 37% of glucose into metab. Deletion of the glkA gene lowered this to 18% and deletion of gltK lowered it to 24%. In the case of gluconic acid, 98% of fed gluconic acid could be fed into ascorbic acid fermn. by deletion of the gntK gene.

L34 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

2002:793598 Document No. 137:309603 Methods for the production of ascorbic acid intermediates in ***Pantoea*** strains. Dodge, Timothy C.; ***Valle, Fernando*** (Genencor International, Inc., USA). PCT Int. Appl. WO 2002081440 A2 20021017, 71 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US10949 20020404. PRIORITY: US 2001-PV281571 20010404; US 2001-PV282277 20010405.

AB The invention provides methods and host cells for the prodn. of ascorbic acid intermediates. The invention also provides host cells having a modification in a polynucleotide that uncouples the catabolic pathway from the oxidative pathway by deleting the encoding for an endogenous enzymic activity that phosphorylates D-glucose at its 6th carbon and/or a polynucleotide that has deleted the encoding for endogenous enzymic activity that phosphorylates D-gluconate at its 6th carbon. Such host cells are used for the prodn. of products, such as, ascorbic acid intermediates. Nucleic acid and amino acid sequences with inactivated enzymic activity which phosphorylates D-glucose at its 6th carbon and inactivated enzymic activity which phosphorylates D-gluconate at its 6th carbon are provided.

L34 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

2002:123238 Document No. 136:182550 Enhanced 2-keto-L-gulonic acid production. ***Kumar, Manoj*** ; ***Valle, Fernando*** ; ***Dartois, Veronique A.*** ; ***Hoch, James A.*** (Genencor International, Inc., USA; Microgenomics, Inc.). PCT Int. Appl. WO 2002012528 A2 20020214, 56 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR,

IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US24327 20010803. PRIORITY: US 2000-633294 20000804; US 2000-677032 20000929.

AB A method for enhancing a host cell's biosynthetic prodn. 2-keto-L-gulonic acid (2-KLG) of is described. Such method comprises selecting a host cell that has an at least partially intracellular synthetic pathway which utilizes 2,5-diketogluconic acid (2,5-DKG) to produce 2-keto-L-gulonic acid; increasing the transport of 2,5-DKG into cell while maintaining the integrity of the cell; culturing the cell to produce 2,5-DKG; and producing 2-KLG. The transport of the 2,5-DKG is increased by transforming into the host cell DNA encoding for one or more transport protein to enhance the supply of the 2,5-DKG into the host cell.

L34 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

2001:800508 Document No. 136:66735 Characterization of sugar mixtures utilization by an Escherichia coli mutant devoid of the phosphotransferase system. Hernandez-Montalvo, V.; ***Valle, F.*** ; Bolivar, F.; Gosset, G. (Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Morelos, 62210, Mex.). Applied Microbiology and Biotechnology, 57(1-2), 186-191 (English) 2001. CODEN: AMBIDG. ISSN: 0175-7598. Publisher: Springer-Verlag.

AB Due to catabolite repression in microorganisms, sugar mixts. cannot be metabolized in a rapid and efficient manner. Therefore, the development of mutant strains that avoid this regulatory system is of special interest to fermn. processes. In the present study, the utilization of sugar mixts. by an Escherichia coli mutant strain devoid of the phosphotransferase system (PTS) was characterized. This mutant can transport glucose (PTS- Glucose+ phenotype) by a non-PTS mechanism as rapidly as its wild-type parental strain. In cultures grown in minimal medium supplemented with glucose-xylose or glucose-arabinose mixts., glucose repressed arabinose- or xylose-utilization in the wild-type strain. However, under the same culture conditions with the PTS- Glucose+ mutant, glucose and arabinose were co-metabolized, but glucose still exerted a partial repressive effect on xylose consumption. In cultures growing with a triple mixt. of glucose-arabinose-xylose, the wild-type strain sequentially utilized glucose, arabinose and finally, xylose. In contrast, the PTS- Glucose+ strain co-metabolized glucose and arabinose, whereas xylose was utilized after glucose-arabinose depletion. As a result of glucose-arabinose co-metab., the PTS- Glucose+ strain consumed the total amt. of sugars contained in the culture medium 16% faster than the wild-type strain. [14C]-Xylose uptake expts. showed that in the PTS- Glucose+ strain, galactose ***permease*** increases xylose transport capacity and the obsd. partial repression of xylose utilization depends on the presence of intracellular glucose.

L34 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

2001:197570 Evaluation of multiple genetic modifications to improve the yield of keto-acid formation in recombinant ***Pantoea*** citrea. Dodge, Tim; Du, Mai; ***Kumar, Manoj*** ; Rashid, Harunur; ***Valle,*** *** Fernando*** (Fermentation Development, Genencor International, Palo Alto, CA, 94304-1013, USA). Abstracts of Papers - American Chemical Society, 221st, BIOT-263 (English) 2001. CODEN: ACSRAL. ISSN: 0065-7727. Publisher: American Chemical Society.

AB ***Pantoea*** citrea has been engineered to allow the formation of 2-keto-L-gulonic acid (KLG), an intermediate in the prodn. of ascorbic acid, and other keto-acids. Several genetic modifications have been implemented to improve the conversion of carbohydrates into keto-acids. These modifications were targeted to 1) remove competing reactions, 2) improve carbon flux into the pathway, and 3) optimize the synthesis of reducing equiv. required for KLG prodn. The evaluation of these genetic changes will be discussed.

L34 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

1997:6065 Document No. 126:30381 Application of glucose transport mutants for production of aromatic pathway compounds. ***Valle, Fernando*** ; Mejia, Naomi; Berry, Alan (Genencor International, Inc., USA; Universidad Nacional Autonoma De Mexico). PCT Int. Appl. WO 9634961 A1 19961107, 52 pp. DESIGNATED STATES: W: CA, FI, JP, MX; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US6284 19960503. PRIORITY: US 1995-435510 19950505.

AB This invention describes methods for enhancing carbon flow into a pathway

of a host cell to enhance the biosynthetic prodn. of compds. therefrom, the host cells being selected based on being phenotypically Pts-/glucose+. Such host cells are capable of transporting glucose without consuming PEP, resulting in conservation of PEP which can be re-directed into the pathway in order to enhance the prodn. of desired compds. along the pathway. Pts-/glucose+ mutants have been shown to be advantageous for the enhanced prodn. of the arom. amino acids. Pts-/glucose+ mutants of Escherichia coli were selected by a novel chemostat procedure. These ptsHIcrr operon mutants transported glucose by the galactose ***permease*** pathway. E. coli transformed with plasmids contg. the aroG and tktA genes and the aroACBLE genes produced 2-3X more Phe and 1.6X more Tyr when the phosphotransferase system was inactivated (i.e. in Pts-/glucose+ mutants).

L34 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

1995:50430 Document No. 122:2352 Identification of a second oligopeptide transport system in Bacillus subtilis and determination of its role in sporulation. Koide, Akiko; ***Hoch, James A.*** (Dept. Molecular and Exptl. Medicine, Scripps Research Inst., La Jolla, CA, 92037, USA). Molecular Microbiology, 13(3), 417-26 (English) 1994. CODEN: MOMIEE. ISSN: 0950-382X.

AB Sporulation in Bacillus subtilis depends on an intact oligopeptide transport system, the Opp system. Mutants in opp sporulate poorly but second-site revertants can be found that restore sporulation and peptide transport. These second-site mutations were found in a second oligopeptide transport system, app, in which the peptide-binding protein, AppA, is mutant owing to a frame-shift mutation, and the revertants restore the original frame. The AppA mutation is present in the 168 strain of B. subtilis. The app operon consists of five genes in the order appD-appF-appA-appB-appC, with the locus designations corresponding to their homolog in the opp operon. Homol. between the app and opp proteins ranges from 54% identity for AppF and OppF, to 22% identity for AppA and OppA. Both the App and Opp ***permease*** systems can transport tetra- and pentapeptides, but tripeptides are not transported by the App system. Strains of the genotype app+opp- are resistant to the tripeptide antibiotic bialaphos. The repaired App system can substitute completely for the Opp system in both sporulation and competence for genetic transformation. The phenotypes raised some speculation about the subunit configuration of the Opp system.

L34 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2003 ACS on STN

1985:573355 Document No. 103:173355 Organization and cloning of a gluconate (gnt) operon of Bacillus subtilis. Fujita, Yasutaro; Nihashi, Junichi; Fujita, Tamie (Sch. Med., Hamamatsu Univ., Hamamatsu, 431-31, Japan). Mol. Biol. Microb. Differ., Proc. Int. Spore Conf., 9th, Meeting Date 1984, 203-8. Editor(s): ***Hoch, James A.; Setlow, Peter*** . Am. Soc. Microbiol.: Washington, D. C. (English) 1985. CODEN: 54BGAE.

AB Several gnt gene mutations that render B. subtilis unable to grow on gluconate [526-95-4] were mapped and characterized. All of the gnt mutations affecting the gluconate ***permease*** [57087-96-4] and kinase [9030-55-1] and the regulation of their biosynthesis were clustered between iol-6 and fdp-74 genes. The gnt-10 mutation and the gnt-23 and gnt-26 mutations were localized in the structural genes of the kinase and ***permease*** , resp., and the gnt-9 mutation in the regulatory region. The 2 structural genes belong to a single transcription unit, the gnt operon. An EcoRI fragment contg. the gnt operon was cloned in temperate phage .vphi.105, and a phys. map was constructed. The phys. location of the mutations coincided with that deduced from their genetic mapping.

	L #	Hits	Search Text	DBs
1	L1	1	DIKETO ADJ2 GLUCONIC ADJ4 PERMEASE	USPAT ; US-PG PUB
2	L2	76	PANTOEA	USPAT ; US-PG PUB
3	L3	932	PERMEASE	USPAT ; US-PG PUB
4	L4	4	L2 AND L3	USPAT ; US-PG PUB

Title: US-09-922-501-7

RESULT 12
AF064803/c
LOCUS AF064803 1776 bp mRNA linear VRT 23-MAY-1998
DEFINITION Ambystoma mexicanum tyrosinase-related-protein-1 (TRP-1) mRNA, complete cds.
ACCESSION AF064803 S77396
VERSION AF064803.1 GI:3152888
KEYWORDS .
SOURCE Ambystoma mexicanum.
ORGANISM Ambystoma mexicanum
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Amphibia; Batrachia; Caudata; Salamandroidea; Ambystomatidae; Ambystoma.
REFERENCE 1 (bases 361 to 1413)
AUTHORS Mason,K.A. and Mason,S.K.
TITLE The identification and partial cloning by PCR of the gene for tyrosinase-related protein-1 in the Mexican axolotl
JOURNAL Pigment Cell Res. 8 (1), 46-52 (1995)
MEDLINE 95312498
PUBMED 7792254
REFERENCE 2 (bases 1 to 1776)
AUTHORS Mason,K.A., Moody,M., Amack,J. and Thibaudeau,G.
TITLE Complete cDNA for Ambystoma mexicanum TRP-1
JOURNAL Unpublished
REFERENCE 3 (bases 1 to 1776)
AUTHORS Mason,K.A.
TITLE Direct Submission
JOURNAL Submitted (12-MAY-1998) Genetics, University of Kansas, 3004 Haworth, Lawrence, KS 66045, USA
COMMENT On May 26, 1998 this sequence version replaced gi:945198.
FEATURES Location/Qualifiers
source 1. .1776
/organism="Ambystoma mexicanum"
/db_xref="taxon:8296"
/dev_stage="larva"
gene 1. .1776
/gene="TRP-1"
CDS 106. .1710
/gene="TRP-1"
/note="bi-nuclear copper binding protein"
/codon_start=1
/product="tyrosinase-related-protein-1"
/protein_id="AAC17168.1"
/db_xref="GI:3152889"
/translation="MLGPATFLPLTAALLLLIPGGRAQFPRQCVTPEALRSGQCCPGL
FPALTPDPADRCGASVGRGRCAPLQVDARPHGPQYPHDGVDDREQWPTRFFNNSCLCA
ENFSGYDCGSCKPGWVGVNCNQRVLAVRRNILDLTAQERRRFIAALDLAKRTTHPHYV
IASRRYAEIMGPDGNSTQFENVSIYNFFVWTHYYSIGKTFLGAGRESFGGIDFSHEGP
AFVTWHRYHLLQLERDMQEMLQDPTFALPYWNFAIGGNECDICTDDFMGARSNFDSIL
LSSNSVFSQWRVLCESLEGYDTLGTICNSTEGGPIRRNPGGNVARPMVQRLPEPQDVA
LCLEVGLFDTPPFYSNSSESFRNTVEGYSEPSGKYDPSVRSLHNLAHLFLNGTGGQTH
VSPNDPIFVLLHTFTDAVFDEWLRRHNADISLYPLENAPIGHNRQYNMVPFWPPVSNN
EMFVTAPESLGYSYEVQWPSRALNFTEIITIAVVAALVLVAVIFAAASCAVHRSRKDD
VHQPLLGEQYPRYSEEYERDASQSAV"
BASE COUNT 408 a 472 c 456 g 440 t
ORIGIN

Query Match 1.2%; Score 20; DB 5; Length 1776;
Best Local Similarity 100.0%; Pred. No. 7.9;
Matches 20; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 472 GCCTGGGCCGTTGTTTCTGT 491
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Db 21 GCCTGGGCCGTTGTTTCTGT 2

Title: US-09-922-501-7

RESULT 14
BE428638/c
LOCUS BE428638 397 bp mRNA linear EST 26-JUL-2000
DEFINITION MTD009.C03F990617 ITEC MTD Durum Wheat Root Library Triticum turgidum subsp. durum cDNA clone MTD009.C03, mRNA sequence.
ACCESSION BE428638
VERSION BE428638.1 GI:9426481
KEYWORDS EST.
SOURCE durum wheat.
ORGANISM Triticum turgidum subsp. durum
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae ; Triticeae; Triticum.
REFERENCE 1 (bases 1 to 397)
AUTHORS Anderson,O.A., Appels,R., Bailey,P., Blake,T., Close,T., Cloutier ,S., Dubcovsky,J., Feuillet,C., Gale,M., Graner,A., Gustafson,P., Herrmann,R.G., Holton,T., Jacquemin,J.M., Jia,J., Joudrier,P., Langridge,P., Lazo,G.R., Lin,J.J., McGuire,P., Ogihara,Y., Pecchioni,N., Qualset,C., Schuch,W., Selvaraj,G., Shariflou,M., Sorrells,M., Warburton,M. and Wenzel,G.
TITLE International Triticeae EST Cooperative (ITEC): Production of Expressed Sequence Tags for Species of the Triticeae
JOURNAL Unpublished (2000)
COMMENT Contact: Joudrier P
INRA, Unite de Biochimie et Biologie Moleculaire des Cereales
2, place VIALA, 34060 Montpellier cedex 01 FRANCE
Tel: 33 4 99 61 23 84
Fax: 33 4 99 61 23 48
Email: joudrier@ensam.inra.fr
International Triticeae EST Cooperative (ITEC)
http://wheat.pw.usda.gov/genome.
FEATURES Location/Qualifiers
source 1. .397
/organism="Triticum turgidum subsp. durum"
/cultivar="Siliana"
/db_xref="taxon:4567"
/clone="MTD009.C03"
/clone_lib="ITEC MTD Durum Wheat Root Library"
/tissue_type="root"
/dev_stage="3-day-old seedling, water-stressed"
/note="Vector: pSPORT1; T7 primers used. See pSPORT1 polylinker site. 0.3-2.0 Kbp average insert size."
BASE COUNT 112 a 97 c 133 g 55 t
ORIGIN

Query Match 1.2%; Score 20; DB 10; Length 397;
Best Local Similarity 100.0%; Pred. No. 31;
Matches 20; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1276 GCACCGCCGGAAGCAGCCGG 1295
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Db 384 GCACCGCCGGAAGCAGCCGG 365

Title: US-09-922-501-7

RESULT 15
BE418965/c
LOCUS BE418965 410 bp mRNA linear EST 24-JUL-2000
DEFINITION WWR017.E8R000101 ITEC WWR Wheat Root Library Triticum aestivum cDNA clone WWR017.E8, mRNA sequence.
ACCESSION BE418965
VERSION BE418965.1 GI:9416811
KEYWORDS EST.
SOURCE bread wheat.
ORGANISM Triticum aestivum
Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; Liliopsida; Poales; Poaceae; Pooideae ; Triticeae; Triticum.
REFERENCE 1 (bases 1 to 410)
AUTHORS Anderson,O.A., Appels,R., Bailey,P., Blake,T., Close,T., Cloutier ,S., Dubcovsky,J., Feuillet,C., Gale,M., Graner,A., Gustafson,P., Herrmann,R.G., Holton,T., Jacquemin,J.M., Jia,J., Joudrier,P., Langridge,P., Lazo,G.R., Lin,J.J., McGuire,P., Ogihara,Y., Pecchioni,N., Qualset,C., Schuch,W., Selvaraj,G., Shariflou,M., Sorrells,M., Warburton,M. and Wenzel,G.
TITLE International Triticeae EST Cooperative (ITEC): Production of Expressed Sequence Tags for Species of the Triticeae
JOURNAL Unpublished (2000)
COMMENT Contact: Schuch W
Zeneca Wheat Improvement Centre, Norwich Research Park
Colney Lane, Norwich NR4 7UH UNITED KINGDOM
Tel: 44 1603 250 2600
Fax: 44 1603 250 699
Email: wolfgang.schuch@aguk.zeneca.com
International Triticeae EST Cooperative (ITEC)
http://wheat.pw.usda.gov/genome.
FEATURES Location/Qualifiers
source 1. .410
/organism="Triticum aestivum"
/cultivar="Novosibirskaya 67"
/db_xref="taxon:4565"
/clone="WWR017.E8"
/clone_lib="ITEC WWR Wheat Root Library"
/tissue_type="root"
/note="M13 Reverse sequencing primer used for 5' end of clone. "
BASE COUNT 87 a 94 c 132 g 97 t
ORIGIN

Query Match 1.2%; Score 20; DB 10; Length 410;
Best Local Similarity 100.0%; Pred. No. 31;
Matches 20; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1276 GCACCGCCGGAAGCAGCCGG 1295
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Db 192 GCACCGCCGGAAGCAGCCGG 173

Title: US-09-922-501-7

4 00

RESULT 5
AC002980/c
LOCUS AC002980 141475 bp DNA linear PRI 26-JAN-1998
DEFINITION Homo sapiens Xp22 BAC 620F15 (Genome Systems BAC library) complete sequence.
ACCESSION AC002980
VERSION AC002980.1 GI:2809267
KEYWORDS HTG.
SOURCE Homo sapiens.
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 141475)
AUTHORS Muzny,D., Arenson,A.D., Brundage,E., Carvelli,K., Chen,E., Di,W., Ding,Y., Dugan,S., Durbin,J., Forcum,J., Ganesh,R., Garcia,C., Goodman,M., Gorrell,J.H., Haywood,M., Jackson,L., Kampal,R., Karpathy,S., Leal,B., Liu,W., Logan,O., Lu,J., Ly,T., Martinez,C., Oswal,G., Perez,L., Rashid,N.D., Rowland,K., Savage,L., Scherer,S.S., Shen,H., Timms,K.M., Todd,J., Vo,Q., Worley,K.C., Yu,W., Chinault,C., Nelson,D. and Gibbs,R.A.
TITLE Direct Submission
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 141475)
AUTHORS Chiu,M.W.
TITLE Direct Submission
JOURNAL Submitted (29-SEP-1997) Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
REFERENCE 3 (bases 1 to 141475)
AUTHORS Worley,K.C.
TITLE Direct Submission
JOURNAL Submitted (26-JAN-1998) Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
COMMENT On Jan 26, 1998 this sequence version replaced gi:2668525.
Sequencing is completed to a minimum standard of double strand coverage with a minimum of 2 clones and 2 reads with no ambiguities or 2 chemistries with a minimum of 2 clones and 3 reads with no ambiguities. If the sequence quality does not meet this standard, it will be indicated in the annotation.

The repeat regions shown were identified using RepeatMasker by Adrian Smit.

Sequence similarities were identified using Powerblast by Jinghui Zhang.

Exon/Intron boundaries of identified genes were chosen if there were canonical splice junctions that maintained sequence continuity across the splice junctions.

FEATURES Location/Qualifiers
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/db_xref="taxon:9606"
/chromosome="X"
/clone="620F15"
/clone_lib="Genome Systems BAC"
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repeat_region complement(4627. .4986)
/rpt_family="THE1C"
repeat_region 4987. .5685
/rpt_family="L1PA13"
STS 6084. .6164
/standard_name="HUMSWX1277, Chr. X, Homo sapiens L42670"
/db_xref="dbSTS:12966"
repeat_region complement(6118. .6186)
/rpt_family="L2"
repeat_region 6246. .6546
/rpt_family="AluSx"
repeat_region 6683. .7105

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                     /rpt_family="MLT1C"
repeat_region        complement(7655. .8161)
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                     /rpt_family="L2"
repeat_region        9192. .9459
                     /rpt_family="L2"
repeat_region        11108. .11226
                     /rpt_family="L2"
repeat_region        complement(11492. .11514)
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repeat_region        complement(11527. .11650)
                     /rpt_family="L1ME1"
repeat_region        complement(11712. .16958)
                     /rpt_family="L1HS"
STS                  12054. .12185
                     /standard_name="A002D07, Chr. -, Homo sapiens G19948"
                     /db_xref="dbSTS:32826"
STS                  13083. .13260
                     /standard_name="STS1-cSRL-31b6-uA/cSRL-31b6-uZ, Chr. -,
                     Homo sapiens G02335"
                     /db_xref="dbSTS:7821"
repeat_region        complement(16954. .17733)
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repeat_region        complement(19811. .20138)
                     /rpt_family="MSTD"
repeat_region        20337. .20529
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repeat_region        21391. .21555
                     /rpt_family="MER5B"
repeat_region        complement(21772. .21897)
                     /rpt_family="MIR"
repeat_region        complement(21898. .22304)
                     /rpt_family="MSTA"
repeat_region        complement(22305. .22361)
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repeat_region        22975. .23489
                     /rpt_family="MLT2CA"
repeat_region        24224. .24529
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repeat_region        24615. .24966
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repeat_region        complement(25229. .25254)
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repeat_region        complement(25975. .26012)
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repeat_region        complement(26284. .26406)
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repeat_region        26408. .26786
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repeat_region        complement(27029. .27076)
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repeat_region        27067. .27193
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repeat_region        27195. .27498
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repeat_region        27503. .28174
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repeat_region        28177. .28467
                     /rpt_family="AluJb"
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                     /rpt_family="FLAM_A"
     repeat_region   complement(31904. .31925)
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     repeat_region   31926. .32180
                     /rpt_family="AluY"
     repeat_region   33697. .33930
                     /rpt_family="AluJo"
     repeat_region   33945. .33983
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                     /rpt_family="MER57_internal"
     repeat_region   complement(35574. .36007)
                     /rpt_family="MER72"
     repeat_region   complement(36076. .36274)
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     repeat_region   complement(38651. .38728)
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     repeat_region   42587. .42935
                     /rpt_family="MLT1A1"
     repeat_region   42940. .42970
                     /rpt_family="(CAT)n"
     repeat_region   complement(43544. .43647)
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     repeat_region   complement(44154. .44197)
                     /rpt_family="(CA)n"
     repeat_region   complement(45254. .45374)
                     /rpt_family="MIR"
     repeat_region   47849. .47971
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     repeat_region   48952. .49170
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     repeat_region   49833. .49931
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     repeat_region   50553. .50975
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     repeat_region   complement(53933. .54449)
                     /rpt_family="MER4B"
     repeat_region   54460. .54760
                     /rpt_family="AluY"
     repeat_region   complement(54772. .54836)
                     /rpt_family="MER4B"
     repeat_region   complement(55390. .55733)

  Query Match             1.3%;  Score 21;  DB 9;  Length 141475;
  Best Local Similarity   100.0%;  Pred. No. 2.7;
  Matches   21;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy        406 CAGGTTCCTGGTGGGCATATC 426
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Db       6733 CAGGTTCCTGGTGGGCATATC 6713
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